

BRIEF COMMUNICATION

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Typing Y Chromosome STR Haplotypes Using Redesigned Primers

REFERENCE: Hou Y, Zhang J, Sun D, Li Y, Wu J, Zhang S, Prinz M. Typing Y chromosome STR haplotypes using redesigned primers. *J Forensic Sci* 2002;47(1):215–217.

ABSTRACT: A panel of Y-specific STR loci, including DYS19, DYS389, DYS390, DYS391, DYS392, and DYS393 was analyzed using horizontal nondenaturing polyacrylamide gel electrophoresis with a discontinuous buffer system (horizontal disk-PAGE). In order to obtain correct results for the larger DYS389 and DYS392 alleles, it was necessary to design new primers that bind closer to the repeat region and lead to a significant reduction of the amplified fragment size. Using the modified primer sets the horizontal disk-PAGE results were consistent with a nondenaturing approach using fluorescent primers and a 377 automated sequencer. The modified procedure also amplifies the second repeat stretch at the duplicated DYS389 locus as a single fragment, which results in an immediate allele identification. The results indicate that horizontal disk-PAGE with silverstaining is a simple approach to type the recommended Y-specific STR markers.

KEYWORDS: forensic science, chromosome Y, horizontal polyacrylamide gel electrophoresis, primers, short tandem repeats, haplotype

Human chromosome Y specific short tandem repeat (Y-specific STR) markers have useful properties for forensic analysis, especially in sexual assault cases where the DNA of the male perpetrator is masked by the victim's DNA and deficiency paternity cases involving male offspring. A panel of Y-specific STR markers (DYS19, DYS389, DYS390, DYS391, DYS392, and DYS393) has been recommended for these and other forensic applications (1–3). We describe an allele typing approach for these Y-specific STR markers using horizontal nondenaturing polyacrylamide gel electrophoresis with a discontinuous buffer system.

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This study was supported by grants from the Alexander von Humboldt Foundation, Germany, and the Chinese Medical Board of New York, USA, and the National Nature Science Foundation as well as the Science Foundation of Sichuan Province, P.R. China.

Received 13 Oct. 2000; and in revised form 19 March 2001; accepted 3 April 2001.

Material and Methods

Blood specimens preserved with EDTA were collected from unrelated males of the Han population in Chengdu, China. Ethnic origin was determined by self declaration. DNA was extracted using the Chelex method (4). Quantification of DNA was undertaken using a primate-specific alpha satellite probe assay (5).

The polymerase chain reactions were carried out using primers for DYS19, DYS389, DYS390, DYS391, DYS392, and DYS393 loci according to Kayser et al. (1). The primers for DYS392 and the DYS389AB loci were modified in order to reduce the amplified fragment size. The new primers for DYS392 locus were redesigned as follows: forward 5'-tcaagtgtttgtatttaaagc-3'; forward 5'-agagggatcattaacctac-3'.

The new primers redesigned by Rolf et al. (6) and de Knijff and Roewer (7) for the DYS389AB locus were modified as noted below, the M13 sequence tag in the 5' region of the reverse primer was not included. Also, we added 4 bases in the 5' region of the reverse primer to optimize the PCR conditions: forward 5'-tcactgttattatctatgttg-3'; forward 5'-ttggatacagaagtaggataa-3'.

Each Y-specific STR locus, except the DYS389AB locus was amplified in a single polymerase chain reaction (PCR). Each PCR setup contained 2 to 40 ng human genomic DNA, 1x Taq buffer, 1.5 mM MgCl₂, 200 μM each nucleotide, 1.5 U Taq polymerase (Promega Corporation, Madison, WI), 0.25 μM each primer in a total volume of 37.5 μL. A total of 30 cycles was carried out in the GeneAmp[®] PCR System 9600 Thermal Cycler (Perkin-Elmer Applied Biosystems Division, Foster City, CA) with denaturation for 50 s at 94°C, annealing for 25 s at 60°C and extension for 25 s at 72°C.

For the DYS389AB locus, a nested PCR strategy was employed. The PCR product of the amplified DYS389 was diluted to 1/1000 and 2 μL of the dilution was used as DNA template for the amplification of the DYS389AB locus. The amplification was then carried out as described above.

The PCR products of each Y-specific STR locus were analyzed using horizontal nondenaturing polyacrylamide gel electrophoresis with a discontinuous buffer system (8). The gels were silverstained (9). Allele determination for each Y-specific STR locus was carried out in comparison to a human allele ladder, which was made in-house according to the recommendations of the International Society of Forensic Haemogenetics (10). In the laboratory in New York, the Y-specific STR alleles were typed using fluorescent labeled primers and two multiplex reactions for DYS19, DYS389,

and DYS390, and DYS391, DYS392, and DYS393 according to Kayser et al. (1). The PCR products were analyzed using denaturing gels and fluorescent detection on an ABI PRISM™ 377 DNA Sequencer (Perkin-Elmer Applied Biosystems Division, Foster City, CA). Allele nomenclature here was based on the allelic ladders kindly provided by Peter de Knijff (The Forensic Laboratory for DNA Research, Leiden University Medical Center, The Netherlands) and Monika Hidding (Institute of Legal Medicine, University of Cologne, Germany). Ten samples were exchanged between Chengdu and New York in order to compare the results for the different detection approaches.

Results and Discussion

Allelic Typing Results

All typing results for DYS19, DYS389CD, DYS390, DYS391, and DYS393 were consistent for both detection methods. However, one allele 18 and an allele 19 at DYS389AB and all alleles 14 at locus DYS392 were misidentified as containing one repeat unit less than the actual number of repeats contained in this amplicon. Since all of the shorter alleles were typed correctly and only the larger alleles sizes showed the delayed migration pattern, the likely explanation was an insufficient ability to separate larger alleles for the nondenaturing horizontal PAGE procedure.

To overcome this problem, new primer sequences that bind closer to the repeat region and lead to a significant reduction of the amplified fragment size were selected. As expected, using the new primers yielded the correct typing results for both loci (Figs. 1 and 2). Now all ten samples shared by both laboratories gave consistent results (Table 1). Typing for all seven Y-specific STR loci with both methods could be shown to be both precise and reliable. It was also demonstrated that reproducible results could be obtained independently. This means the less expensive horizontal nondenaturing polyacrylamide gel electrophoresis followed by silverstaining can be reliably used for allele identification of these Y-STR markers. The method was successfully used to generate a database of Chinese Han haplotypes. The complete population data set can be accessed at <http://www.legalmed.org/dna/YSTR7.htm>.

Nomenclature of Alleles at DYS389

As shown in Fig. 3, the DYS389 locus contains a total of four tandem repeat structures, coded A, B, C, and D according to de Knijff et al. (2) or m, n, p, q according to Rolf et al. (6). Allele detection with the previously described primers for DYS389 (1) will result in two PCR fragments since the forward primer anneals twice (Fig. 3). The shorter PCR fragment containing C and D was named DYS389I and the longer one including A, B, C, and D was

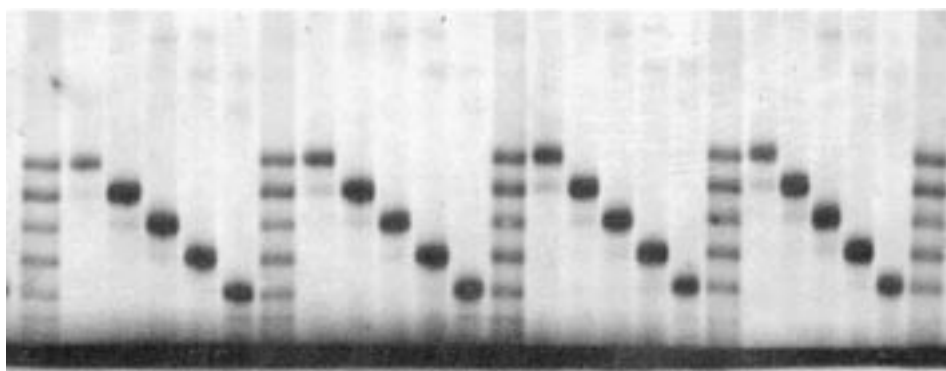


FIG. 1—DYS389AB results in a horizontal nondenaturing gel. DYS389AB human allelic ladder composed of alleles with 15, 16, 17, 18, and 19 repeats. Anode on the bottom. Lane 1, 7, 13, 19, 25: human allelic ladder; Lane 2, 8, 14, 20: allele 19; Lane 3, 9, 15, 21: allele 18; Lane 4, 10, 16, 22: allele 17; Lane 5, 11, 17, 23: allele 16; and Lane 6, 12, 18, 24: allele 15.

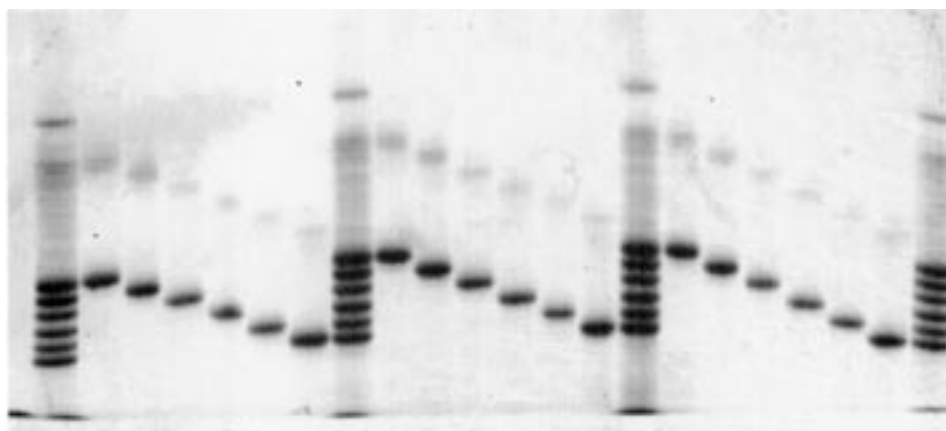


FIG. 2—DYS392 results in a horizontal nondenaturing gel. DYS392 human allelic ladder composed of alleles with 10, 11, 12, 13, 14, 15 repeats. Anode on the bottom. Lane 1, 8, 15, 22: human allelic ladder; Lane 2, 9, 16: allele 15; Lane 3, 10, 17: allele 14; Lane 4, 11, 18: allele 13; Lane 5, 12, 19: allele 12; Lane 6, 13, 20: allele 11; and Lane 7, 14, 21: allele 10.

TABLE 1—Typing results for the different approaches.

Typing Locus	CH1	CH2	CH3	CH4	CH5	CH6	CH8	CH9	CH10	CH11
DYS19*	15	18	15	15	17	15	15	15	15	15
DYS19†	15	18	15	15	17	15	15	15	15	15
DYS389CD*	9	9	9	11	11	11	11	11	11	11
DYS389CD†	9	9	9	11	11	11	11	11	11	11
DYS389AB*	18	18	18	17	17	18 [§]	17	17	17 [§]	17
DYS389AB†	18	18	18	17	17	19	17	17	18	17
DYS389AB‡	18	18	18	17	17	19	17	17	18	17
DYS390*	23	23	23	24	23	23	22	24	23	23
DYS390†	23	23	23	24	23	23	22	24	23	23
DYS391*	10	10	10	10	9	10	10	11	10	10
DYS391†	10	10	10	10	9	10	10	11	10	10
DYS392*	12	13	13 [§]	13	11	13 [§]	10	11	13 [§]	11
DYS392†	12	13	14	13	11	14	10	11	14	11
DYS392‡	12	13	14	13	11	14	10	11	14	11
DYS393*	12	12	13	14	13	13	15	13	13	15
DYS393†	12	12	13	14	13	13	15	13	13	15

* PCR with the previously described primers, typing with horizontal disk-PAGE.
 † PCR with the previously described primers, typing with ABI Prism™ 377 DNA Sequencer.
 ‡ PCR with the modified primers, typing with horizontal disk-PAGE.
 § Miscalled alleles.

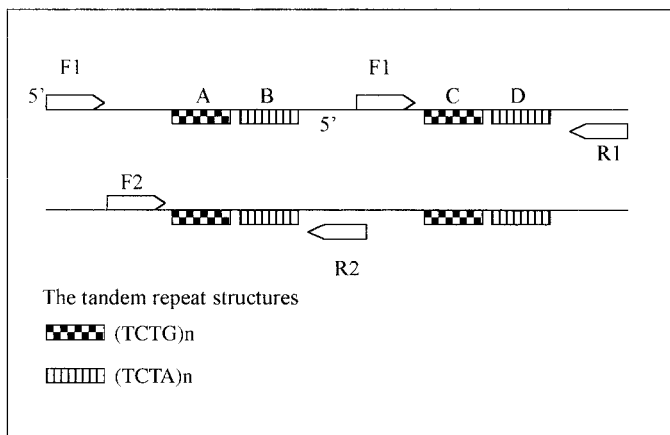


FIG. 3—The tandem repeat structures of DYS389 and different primers for PCR. F1: previously described forward primer; R1: previously described reverse primer; F2: modified forward primer; and R2: modified reverse primer.

named DYS389II according to Kayser et al. (1) and de Knijff et al. (2). Unfortunately, this nomenclature led to counting the variable CD stretch twice and there is no straightforward allele identification of the number of repeats for the AB region. In order to identify the true allele for DYS389AB, one has to subtract the repeat number present at DYS389I (or CD) from the DYS389II result. This is not necessary using our nested PCR approach. The compound character of the DYS389II allele is no problem for forensic casework where the comparison is made based on the complete haplotype but it does cause problems for population studies. For example in our population sample, DYS389II was observed to have seven alleles, but the region A and B had only five alleles when it was amplified as a single fragment using the modified primers (Fig. 3).

DYS389 nomenclature was discussed at the II International Forensic Y-STR user workshop (Berlin 2000) and it was suggested to replace the current DYS389I and II locus designations with the terms DYS389AB for the longer and DYS389CD for the shorter al-

lele range. It should be mentioned that Rolf et al. (6) further subdivide the DYS389 stretches based on the two types of repeat motifs and developed multiple nested reactions in order to type the additional variability. This implies that the nomenclature of the DYS389 alleles could be made more complex, even though the subdivision does not seem to be necessary for most applications. However, using the nomenclature of DYS389AB and DYS389CD already resolves the ambiguities of the DYS389II allele identification.

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